YALE UNIVERSITY
NEW HAVEN · CONNECTICUT
DEPARTMENT OF BIOCHEMISTRY

STERLING HALL OF MEDICINE
333 CEDAR STREET

February 26, 1957

Dear Josh,

Thank you for the reprints and, especially, for your note. I was delighted to find that someone remembers that strain SF work, and I do appreciate your comments.

You may be amused to hear that only last Thursday I gave a report to our departmental lunch seminar on my old data "reconsidered" in the light of your Proceedings paper and Park's subsequent paper in Science. We also came to the conclusion that penicillin might be blocking the active uptake of glycine by the cells. However, the data might also mean that penicillin inhibits the mechanism of incorporating free glycine into cell wall peptide and that glycine in peptide already linkage gets incorporated by a penicillin-insensitive transfer. There isn't any way to decide this point at present.

I have some data on the effect of penicillin on K12. It's not really "clean", and I've never considered it good enough to publish, but some of it may interest you. I gathered from your paper that you've had difficulty working with coli in synthetic medium, and I'm not surprised.

K12 grows fairly well in the medium I used for strain SF (use either 5 g NaCl or 6.4 g KCl) with L-alanine ([redacted] 0.01 moles per liter) as the sole C- and N-source. The effects of penicillin, glycine, glycylglycine, and alanylalanine under these conditions are illustrated by the following table.

Compound in moles/l	Ala (0.01)	Ala + Gly (each 0.01)	Ala (.01) + Glygly (0.005)	Alaala (0.005) + Gly (0.01)
	Optical density reading at 30 hours (i.e., measure of lag time) when tubes contained at 0 hours 3×10^7 cells/ml.			
Control	.126	.156	.161	.172
+ 10 Oxford units of pen. per ml.	.066	.071	.114	.096
+ 20 Oxford units/ml.	.066	.009	.100	.014

Actually, the inhibition depends on the amount of glycine added, the more glycine the more inhibition. I came to the conclusion, on the basis of viable cell counts, that the apparant inhibition by penicillin was due, in large part, to killing of cells. Moreover, if the reponses to ala + glycine with and without 20 units of penicillin are compared, the kill due to penicillin occurs at just a about the time when active cell multiplication starts in the absence of penicillin.

From my experience with K12 and some of its mutants (58, 679), I'd say that the various strains may vary somewhat in their sensitivity to penicillin. K12 is always MORE sensitive to penicillin in that SF medium with alanine as C- and N- source than it is in the presence of glucose and ammonium salts. However, the concentration of inorganic salts must be relatively high (just as I reported for strain SF). That salt effect is probably on the binding of penicil-

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lin by the cells. For example, when strain SF is preincubated in SF minimal (devoid of C- and N-source) containing penicillin for 5 hours, and then transferred to fresh medium (no penicillin) with glycine + leucine, the cells show inhibited ability to grow. As you would expect there isn't any kill during the preincubation period. If the preincubation is carried out in SF minimal devoid of NaCl (or KCl), on transfer the cells behave as though they'd never been exposed to penicillin. I don't know if this is true of K12.

By the way, the reason I asked you for the glycine auxotroph way back in 1952 was to use it for penicillin studies. Such studies have never been done - your auxotrophs turned out to be full of other interesting characteristics. I think I may have written you that the serine auxotroph (1977) will grow on glycine peptides in the absence of serine, and, given a long enough incubation time, it even grows on free glycine. In fact, if the asparagine is omitted from Tatum's basal medium (or if glutamic acid is added to Tatum's medium) this so-called serine auxotroph responds quite well to free glycine. This is a pretty problem in glycine penetration all be itself, and it takes on added interest now in relation to the problem of penicillin effect. I hope to get back to this some day. Just now we are revising our book (poor fools, we!), and I haven't done any lab work in months.

Will you and Esther be going to the Federation meetings in Chicago in April? It's been much too long since we've seen you, and I hope Chicago is near enough to Madison for you to make the trip.

With best regards from Joe,

Sincerely,

Topsy

P.S. Excuse this terrible typing. As W.M Clark once said, my typewriter doesn't know how to spell - and I don't give it much help.